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Data Sheet

Myc Reporter Kit (Myc signaling pathway)
Catalog #: w70530

Background

The Myc signaling pathway plays an important role in cell proliferation, differentiation, transformation and apoptosis. The c-Myc protein is a transcription factor that heterodimerizes with Max to regulate Myc signaling pathway responsive genes. Myc mutations have been linked to the development of a number of human cancers, including Burkitt's lymphoma, cervical, ovarian, breast, lung and pancreatic carcinoma, making Myc a promising therapeutic target for cancer treatment.

Description

The Myc Pathway Reporter kit is designed for monitoring the activity of the Myc signaling pathway in cultured cells. The kit contains a transfection-ready expression vector for c-Myc and Myc luciferase reporter vector. Inside the cells, c-Myc will bind to Max, translocate to the nucleus, and induce expression of the Myc luciferase reporter vector. This reporter contains the firefly luciferase gene under the control of multimerized Myc responsive elements located upstream of a minimal promoter. The Myc reporter is premixed with constitutively-expressing *Renilla* (sea pansy) luciferase vector, which serves as an internal positive control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, but without any additional response elements. The negative control is critical for determining pathway-specific effects and background luciferase activity.

Applications
- Monitor Myc signaling pathway activity.
- Screen activators or inhibitors of the Myc signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the Myc pathway.

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	Myc luciferase reporter vector + constitutively-expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively-expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
c-Myc Expression Vector (Component C)	c-Myc expression vector	500 µl (100 ng DNA/µl)	-20°C
Negative Control Expression Vector (Component D)	Empty expression vector without c-Myc	500 µl (100 ng DNA/µl)	-20°C

These vectors are designed for use in transient transfections. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagents for mammalian cell line. We use Lipofectamine™ LTX with PLUS™ reagent (Life Technologies #15338100) or Lipofectamine™ 2000 (Life Technologies # 11668027). However, other transfection reagents should work equally well.
- Opti-MEM I Reduced Serum Medium (Life Technologies #31985-062)
- Dual luciferase assay system:
  Dual-Glo® Luciferase Assay System (Promega #E2920): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
  OR
  Dual-Luciferase® Reporter Assay System (Promega #E1910): This system requires a cell lysis step. It is ideal for luminometers with automated injectors.
- Luminometer

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Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HCT116 cells using Lipofectamine LTX in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine LTX, follow the manufacturer's transfection protocol. Transfection conditions should be optimized according to the cell type and study requirements.
Note: This protocol is for HCT116 cells. For HEK293 cells, we use Lipofectamine™ 2000 according to manufacturer's protocol.

All amounts and volumes in the following setup are given on a per well basis.

1. One day before transfection, seed cells at a density of ~ 30,000 cells per well in 100 µl of growth medium so that cells will be 80-90% confluent at the time of transfection.

2. Next day, for each well, prepare complexes as follows:

   a. Dilute DNA mixtures in 20 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of the following combinations:
      - 1 µl of **Reporter** (component A); in this experiment, the control transfection is 1 µl of **Negative Control Reporter** (component B).
      - 1 µl of **Reporter** (component A) + **c-Myc Expression Vector** (component C); in this experiment, the control transfections are: 1 µl of **Reporte**r (component A) + **Negative Control Expression Vector** (component D), 1 µl of **Negative Control Reporter** (component B) + **c-Myc expression vector** (component C), and 1 µl of **Negative Control Reporter** (component B) + **Negative Control Expression vector** (component D).
      - 1 µl of **Reporter** (component A) + specific siRNA; in this experiment, the control transfections are: 1 µl of **Reporte**r (component A) + negative control siRNA, 1 µl of **Negative Control Reporter** (component B) + specific siRNA, and 1 µl of **Negative Control Reporter** (component B) + negative control siRNA.

   Note: we recommend setting up each condition in at least triplicate, and prepare transfection cocktail for multiple wells to minimize pipetting errors.

   b. Add 0.1 µl of PLUS reagent to diluted DNA, mix gently. Incubate 10 minutes at room temperature.

   c. After the 10 minute incubation, add 0.6 µl Lipofectamine LTX reagent to the diluted DNA. Mix gently and incubate for 25 minutes at room temperature.

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3. Add the 20 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37℃ in a 5% CO_2 incubator. After ~24 hours of transfection, change medium to fresh growth medium. ~48 hours after transfection, perform the dual luciferase assay following the manufacturer's protocol.

To study the effect of activators / inhibitors on the pathway, treat the cells with tested activator/inhibitor after 24 hours of transfection. Perform dual luciferase assay ~48 hours after transfection.

Sample protocol to determine the effect of human c-Myc on Myc reporter activity in HCT116 and HEK293 cells

Additional materials required in this experiment setup:

- HCT116 growth medium: McCoy's 5a medium + 10% FBS + 1% penicillin/ streptomycin. HCT116 is a human colon cancer cell line with a mutated β -catenin which leads to the accumulation of β-catenin and constitutive activation of the Myc signaling pathway.
- HEK293 growth medium: MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) + 10% FBS, 1% non-essential amino acids, 1 mM sodium pyruvate + 1% penicillin/streptomycin (Pen/Strep)
- Assay medium: Opti-MEM I (Life Technologies #31985-062) + 0.5% FBS + 1% Non-essential amino acids + 1 mM sodium pyruvate + 1% Pen/Strep

1. One day before transfection, seed HCT116 or HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate (Corning #3610) in 100 µl of growth medium. Incubate cells at 37°C in a CO_2 incubator overnight.

2. Next day, transfect 1 µl of **Reporter** (component A) or 1 µl **Negative Control Reporter** (component B) with 1 µl of **c-Myc Expression Vector** (component C) or **Negative Control Expression Vector** (component D) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, change medium in wells to 50 µl of fresh assay medium. Add 50 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate cells at 37°C in a CO_2 incubator for ~24 hours.

4. After ~48 hours of transfection, perform dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 50 µl of Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Add 50 µl of Stop & Glo reagent per well. Rock at room temperature for ~15 minutes and measure *Renilla* luminescence.

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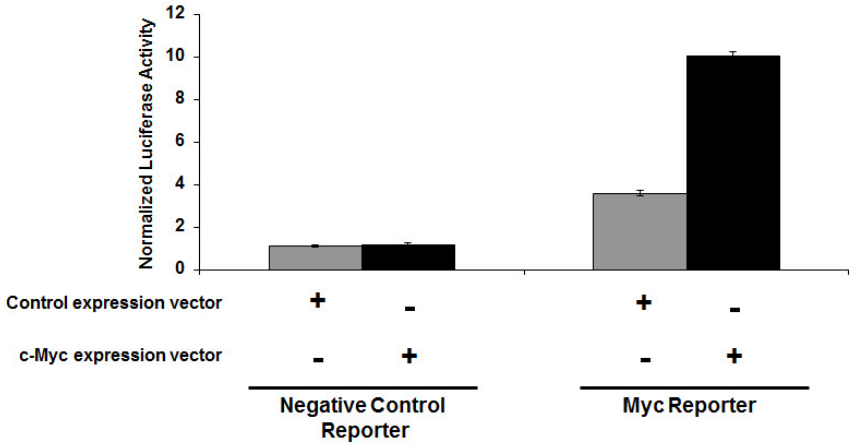
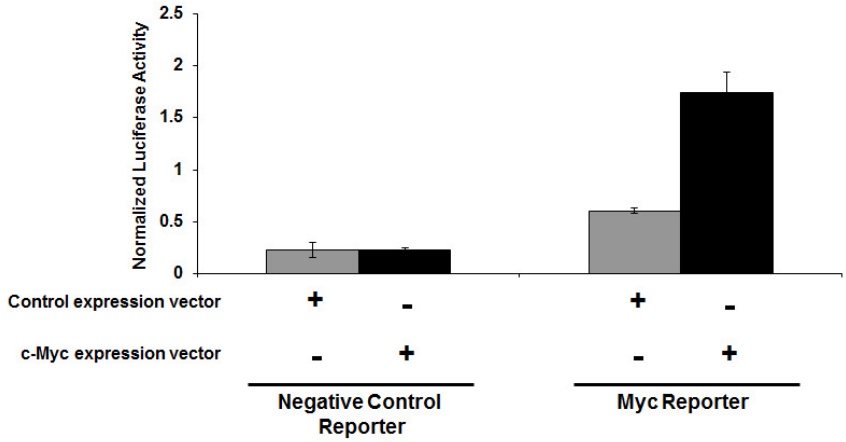
5. To obtain the normalized luciferase activity for Myc reporter, subtract background luminescence, and then calculate the ratio of firefly luminescence from Myc reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 1. Activation of the Myc luciferase reporter by human c-Myc. The results are shown as normalized Myc luciferase reporter activity. Normalized luciferase activity is determined by dividing luciferase data with *Renilla* luciferase data. **(A)** HCT116 cells **(B)** HEK293 cells

A.



B.



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Sample protocol to determine the effect of inhibitor ICG-001 on Myc reporter activity in HCT116 cells.

Additional materials required in this experiment setup

- HCT116 growth medium
- ICG-001 (Selleckchem #S2662): wnt-β-catenin pathway inhibitor. Myc can be activated by wnt pathway.
- Assay medium

1. One day before transfection, seed HCT116 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate (Corning #3610) in 100 µl of growth medium. Incubate cells at 37°C in a CO_2 incubator overnight.

2. Next day, transfect 1 µl of Myc luciferase reporter (component A) into cells following the procedure in "Generalized Transfection and Assay Protocols"**.**

3. After ~24 hours of transfection, remove medium in wells, add threefold serial dilution of ICG-001 in 50 µl of assay medium to inhibited wells; add 50 µl of assay medium to control wells; add 50 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

4. Incubate at 37°C in a CO_2 incubator for 18 hours.

5. Perform dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 50 µl of Luciferase reagent per well and rocking at room temperature for ~15 minutes and measure firefly luminescence using a luminometer. Add 50 µl of Stop & Glo reagent per well and rocking at room temperature for ~15 minutes and measure *Renilla* luminescence.

6. To obtain the normalized luciferase activity for Myc reporter, subtract background luminescence then calculate the ratio of firefly luminescence from the Myc reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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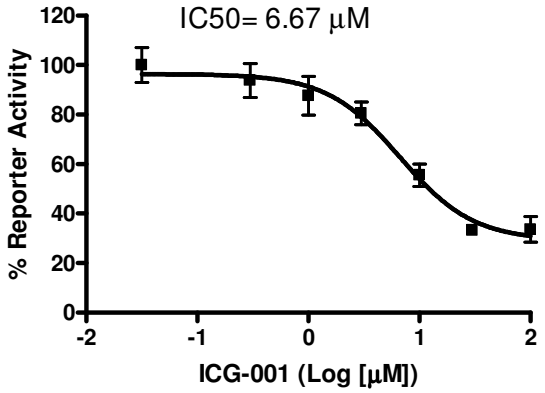
Figure 2. Dose response inhibition of constitutively active Myc reporter activity to inhibitor ICG-001 in HCT116 cells. The results are shown as percentage of Myc reporter activity. The normalized luciferase activity for Myc reporter transfected cells without ICG-001 treatment was set at 100%. The IC_{50} of ICG-001 is ~ 6.67 µM.



References

Pelengaris S, *et al*. (2002) c-MYC: more than just a matter of life and death. *Nat. Rev. Cancer*. **2(10):** 764-76.